WHAT GOES WRONG DURING EARLY DEVELOPMENT OF ARTIFICIALLY REPRODUCED EUROPEAN EEL (*Anguilla anguilla*)? CLUES FROM THE LARVAL TRANSCRIPTOME AND GENE EXPRESSION PATTERNS.

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Introduction

Closing the life cycle of the European eel in captivity is urgently needed to gain perspective for the commercial production of juvenile glass eels. Larvae are produced weekly at our facilities but large variations in larvae mortality are observed during the first week after hatching. Although much effort has been devoted on investigating ways to prevent early larval mortality, it remains unclear what the causes are. The aim of this study was to perform a transcriptomic study on European eel larvae in order to identify genes and physiological pathways that are differentially regulated in the comparison non-viable *vs*. viable larvae.

Material and methods

Larvae collected at 1 day post-hatch (dph) from batches that survived for at least a week were classified as viable larvae, while those from batches that survived less than 3dph were classified as non-viable larvae. RNA was isolated from these samples, RNA-seq was performed and differentially expressed genes were analysed between non-viable *vs*. viable larvae. The major histocompatibility complex class-I (*mhc1*) gene, M-protein (*myom2*), the dopamine 2B receptor (*d2br*), the melatonin receptor (*mtr1*) and heat-shock protein beta-1 (*hspb1)* showed strong differential expression in the RNA-seq data. Consequently, expression patterns of these genes were investigated in 1, 8 and 15dph larvae (Fig. 1) by RT-PCR to further comprehend their role during early ontogeny.

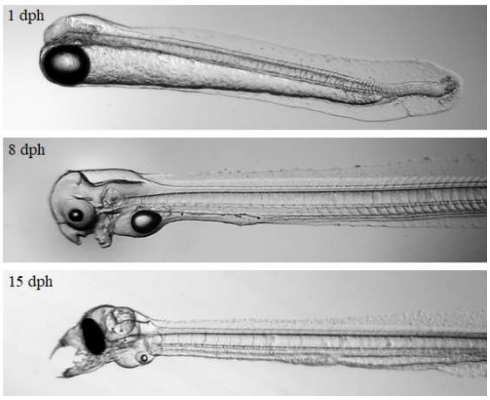

Figure 1. Larvae at 1, 8 and 15dph. At 1dph, larvae hang vertically in the water column and have yolk-sac reserves with large oil droplet. At 8dph, larvae start swimming and develop lower and upper jaws. At 15 dph, the yolk-reserves are almost depleted and the protruding teeth are formed which marks the start of exogeneous feeding.

Results and discussion

Expression of genes involved in inflammation and host protection were higher in non-viable *vs*. viable larvae suggesting that non-viable larvae suffered from microbial infection. Expression of genes involved in osmoregulation were higher in non-viable *vs*. viable larvae implying that non-viable larvae were possibly damaged and tried to maintain homeostasis by strong osmoregulatory adaptation. Myogenesis, neural and sensory development were reduced in non-viable *vs*. viable larvae, probably because non-viable larvae invested energy in the immune response and homeostasis at the cost of developmental processes. Expression of *d2br*, *hspb1* and *mtr1* increased during ontogeny which may reflect the increase in movement at the start of active swimming (8 dph) and feed searching behaviour (15 dph). Expression of *mhc1* was highly expressed at all time points reflecting an active immune system immediately after hatching. Expression of *myom2* decreased during ontogeny reflecting the investment in growth that decreased in line with the consumption of yolk-sac reserves.

In conclusion, larvae exhibit immune competency. Non-viable larvae initiated an immune response but suffered from microbial infection. Non-viable larvae tried to maintain ionic and water homeostasis by strong osmoregulatory adaptations. Microbial control and salinity reduction might benefit eel larvae in terms of lower mortality and improved development by lowering the costs of immune functioning and osmoregulation.

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